

Complete Listing of Claims Pursuant to 37 C.F.R. §1.121

This listing of claims will replace all prior versions, and listings, of claims in the application:

57. [currently amended] A method for ~~sequence analysis of~~ sequencing one or more target nucleic acids present in one or more biological samples, said method comprising the steps of:

- (a) deriving from one or more biological samples the one or more target nucleic acids;
- (b) subjecting the one or more target nucleic acids obtained from step (a) to ~~a set of~~ two or more separate base-specific, sequence-specific or site-specific complementary cleavage reactions, wherein each cleavage reaction generates a non-ordered set of fragments;
- (c) analyzing the sets of non-ordered fragments obtained from step (b) by mass spectrometry; and,
- (d) performing a systematic computational analysis on the mass spectra obtained from step (c) to analyse the sequence of said target nucleic acid,

wherein said complementary cleavage reactions refer to target nucleic acid digestions characterized by varying specificity and/or to digestion of alternative forms of the target sequence.

58. [previously presented] The method according to claim 57 wherein the one or more biological samples are derived from organism selected from the group consisting of eukaryotes, prokaryotes, and viruses.

59. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids are selected from the group consisting of single stranded DNA, double stranded DNA, cDNA, single stranded RNA, double stranded RNA, DNA/RNA hybrid, and DNA/RNA mosaic nucleic acid.

60. [previously presented] The method according to claim 57 wherein one or more target nucleic acids are derived by one or more consecutive amplification procedures selected from the group consisting of in vivo cloning, polymerase chain reaction (PCR), reverse

transcription followed by the polymerase chain reaction (RT-PCR), strand displacement amplification (SDA), and transcription based processes.

61. [currently amended] The method according to claim 4 60 wherein the one or more amplified target nucleic acids are transcripts generated from a single stranded or a double stranded target nucleic acid by a process comprising the steps of:

- (a) linking operatively a transcription control sequences to the one or more target nucleic acids; and
- (b) transcribing one or both strands of the one or more target nucleic acid of step a) using one or more RNA polymerases that recognize the transcription control sequence on the one or more target nucleic acids.

62. [previously presented] The method according to claim 61 wherein said transcriptional control sequences are operatively linked to one or more target nucleic acids by PCR amplification using primers that incorporate the transcriptional control sequences as 5'-extensions.

63. [previously presented] The method according to claim 61 wherein the transcription control sequence is selected from the group consisting of eukaryotic transcription control sequences, prokaryotic transcription control sequences, and viral transcription control sequences.

64. [previously presented] The method according to claim 63 wherein the prokaryotic transcription control sequence is selected from the group consisting of T3, T7, and SP6 promoters.

65. [previously presented] The method according to claim 64 wherein the RNA polymerases which utilize the T3, T7, or SP6 promoters are either wild type or mutant RNA polymerases, the mutant polymerases being capable of incorporating into the transcript non-canonical substrates with a 2'-deoxy, 2'-O-methyl, 2'-fluoro or 2'-amino substituent.

66. [previously presented] The method according to claim 65 wherein the mutant RNA polymerase is either T7 or SP6 mutant polymerase.

67. [previously presented] The method according to claim 57 wherein the derived target nucleic acid incorporates one or more nucleosides that are modified on the base, the

sugar, and/or the phosphate moiety, wherein the modifications alter the specificity of cleavage by the one or more cleavage reagents and/or the mass and/or the length of the cleavage products.

68. [previously presented] The method according to claim 67 wherein the modification is introduced through the enzymatic incorporation of modified deoxynucleoside triphosphates, modified ribonucleoside triphosphates, and/or modified dideoxynucleoside triphosphates; or wherein the modification is introduced chemically, or wherein the modification is introduced through a combination of both methods.

69. [previously presented] The method according to claim 67 wherein the modification consists of a 2'-deoxy, 2'-O-methyl, 2'-fluoro or 2'-amino substituent on the nucleotide triphosphates.

70. [previously presented] The method according to claim 67 wherein the modification consists of phosphorothioate internucleoside linkages or phosphorothioate internucleoside linkages further reacted with an alkylating reagent.

71. [previously presented] The method according to claim 67 wherein the modification consists of a methyl group on C5 of the uridine-5'-monophosphate subunits.

72. [previously presented] The method according to claim 67 wherein the modification consists of nucleotides that incorporate alternative isotopes.

73. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids of step (a) are purified prior to cleavage.

74. [previously presented] The method according to claim 73 wherein said purification is achieved through immobilization or by chromatography.

75. [previously presented] The method according to claim 57 wherein the complementary cleavage reactions are selected from the group consisting of enzymatic cleavage, chemical cleavage, and physical cleavage.

76. [previously presented] The method according to claim 75 wherein the complementary cleavage reactions are characterized by a relaxed mono-nucleotide, mono-nucleotide, relaxed di-nucleotide, or di-nucleotide specificity.

77. [previously presented] The method according to claim 75 wherein the one or more target nucleic acids are subjected to chemical digestion reaction consisting of treatment with alkali or with reagents used in the Maxam & Gilbert sequencing method.

78. [previously presented] The method according to claim 75 wherein the one or more target nucleic acids are subjected to enzymatic cleavage reaction using one or more enzymes selected from the group consisting of endonucleases and exonucleases.

79. [previously presented] The method according to claim 78 wherein the one or more target nucleic acids are subjected to enzymatic cleavage reaction using one or more endonucleases, selected from the group consisting of restriction enzymes, RNA endonucleases, DNA endonucleases and non-specific phosphodiesterases.

80. [previously presented] The method according to claim 79 wherein the one or more endonucleases are one or more selective or non-selective RNA endonucleases, selected from the group consisting of the G-specific T1 ribonuclease, the A-specific U2 ribonuclease, the A/U specific phyM ribonuclease, the U/C specific ribonuclease A, the C-specific chicken liver ribonuclease (RNaseCL3) and cusativin, non-specific RNase-I, and pyrimidine-adenosine preferring RNases isolated from *E. coli*, *Enterobacter sp.*, or *Saccharomyces cerevisiae*.

81. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids are phosphorothioate-modified single stranded DNA or RNA, and wherein the cleavage reactions are performed with the nuclease P1.

82. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids are mosaic RNA/DNA nucleic acids or modified mosaic RNA/DNA nucleic acids, prepared with mutant polymerases, and wherein the cleavage reagents are RNA endonucleases, DNA endonucleases or alkali.

83. [previously presented] The method according to claim 57 wherein the one or more target nucleic acids are transcripts, modified transcripts, mosaic RNA/DNA transcripts or modified mosaic RNA/DNA transcripts, prepared with wild type or mutant RNA polymerases, and wherein the cleavage reagents are one or more selective or non-selective RNA endonucleases or alkali.

84. [currently amended] The method according to claim 57 wherein the one or more target nucleic acids are mosaic RNA/DNA transcripts that incorporate either dCMP, dUMP or dTMP, prepared with mutant T7 or SP6 polymerase, and wherein the cleavage reagent is a pyrimidine-specific RNase, ~~such as RNase-A.~~

85. [previously presented] The method according to claim 57 wherein the set of non-ordered fragments of step (b) is additionally purified using an ion exchange beads.

86. [previously presented] The method according to claim 57 wherein the set of non-ordered fragments of step (b) is spotted onto a solid support.

87. [previously presented] The method according to claim 86 wherein said solid support is chosen from a group consisting of solid surfaces, plates and chips.

88. [previously presented] The method according to claim 57 wherein the mass spectrometric analysis of the nucleic acid fragments is performed using a mass spectrometric method selected from the group consisting of Matrix-Assisted Laser Desorption/Ionization-Time-of-flight (MALDI-TOF), Electrospray-Ionization (ESI), and Fourier Transform-Ion Cyclotron Resonance (FT-ICR).

89. [currently amended] The method according to claim 57, wherein said method is used for sequence analysis re-sequencing of one or more target nucleic acids for which a reference nucleic acid sequence is known; said method comprising an additional step wherein the one or more mass spectra of the non-ordered fragments obtained in step c) are compared with the known or predicted mass spectra for a reference nucleic acid sequence, and deducing therefrom, by systematic computational analysis, all or part of the nucleotide sequence of the one or more target nucleic acids, and comparing the deduced nucleic acid sequence with the reference nucleic acid to determine whether the one or more target nucleic acids have the same sequence or a different sequence from the reference nucleic acid.

90. [previously presented] The method according to claim 89 wherein the nucleic acid sequence difference that is determined is a deletion, substitution, insertion or combinations thereof.

91. [previously presented] The method according to claim 90 wherein the nucleic acid sequence difference is a Single Nucleic Polymorphism (SNP).

92. [currently amended] ~~Use of a~~ The method according to claim 89 wherein said method identifies for scoring known as well as unknown nucleotide sequence variations of said one or more target nucleic acids present in said one or more biological samples.

93. [currently amended] ~~Use~~ The method according to claim 92 wherein ~~the~~ determination of said known or unknown nucleotide sequence variations allows the identification of the various allelic sequences of a certain region/gene, the scoring of disease-associated mutations, the detection of somatic variations, or studies in the field of molecular evolution.

94. [currently amended] ~~Use of a~~ The method according to claim 86 ~~57~~ wherein the spectra obtained for one or more target nucleic acids are compared with the mass spectra predicted for a plurality of reference nucleic acids thereby identifying/detecting one or more target nucleic acids in one or more biological samples.

95. [currently amended] ~~Use~~ The method according to claim 94 wherein said method produces to determine the an expression profile of ~~one or more target nucleic acids in~~ one or more biological samples.

96. [currently amended] A method according to claim 57 for ~~sequence analysis~~ sequencing of one or more target nucleic acids of unknown sequence present in one or more biological samples, said method comprising the steps of:

- (a) deriving from one or more biological samples one or more target nucleic acids in a single stranded form;
- (b) subjecting the one or more target nucleic acids obtained from step (a) to a set of four separate base-specific complementary cleavage reactions, wherein each cleavage reaction generates a non-ordered set of fragments;
- (c) analyzing the sets of non-ordered fragments obtained from step (b) by mass spectrometry;
- (d) performing a systematic computational analysis on the mass spectra obtained from step (c) to assemble the sequence of said target nucleic acid; and,

(e) optionally, if the sequence is not uniquely defined after step (d), repeating steps (a) through (d), thereby generating modified forms of said target nucleic acid and/or different portions of said target nucleic acid, and performing supplementary mono- and/or di-nucleotide specific cleavage reactions rendering supplementary sets of non-ordered fragments until the combined data converge into a unique sequence solution, wherein said complementary cleavage reactions refer to target nucleic acid digestions characterized by varying specificity and/or to digestion of alternative forms of the target sequence.

97. [canceled] Use of a method according to claim 96, for the sequence analysis of one or more target nucleic acids of unknown sequence present in one or more biological samples is unknown prior to performing said method.

98. [canceled] Use of a method according claim 96 for the sequence determination of one or more target nucleic acids of unknown sequence present in one or more biological samples.

99. [currently amended] ~~Use of a~~ The method according to claim 89 for 92 wherein said method provides genome wide genotyping of one or more biological samples.

100. [currently amended] A kit for ~~sequence analysis~~ mass spectrometry re-sequencing according to a method of claim ~~57~~ 89 of one or more target nucleic acids for which a reference nucleic acid sequence is known in one or more biological samples using mass spectrometry, the kit comprising:

- (a) one or more nucleotide triphosphates;
- (b) one or more polymerases;
- (c) one or more nucleic acid cleaving agents; and;
- (d) one or more sets of reference nucleic acids for which the nucleic acid sequence is known;
- (e) ~~optionally~~, reagents to purify the target nucleic acid;
- (f) ~~optionally~~, ion exchange beads in order to purify the non-ordered set of fragments;
- (g) ~~optionally~~, a solid support suitable for use in mass spectrometry analysis whereon the non-ordered set of fragments may be spotted; and,

- (h) ~~optionally~~, computer software for comparing the mass spectra of the one or more target nucleic acid with the mass spectra of the reference nucleic acid and deducing therefrom the nucleic acid sequence of the target nucleic acid.

101. [canceled] Use of a kit according to claim 100 for analyzing the sequence of, for determining sequence differences in, for scoring known as well as unknown nucleotide sequence variations in, for detecting/identifying, or, for performing genome wide genotyping using one or more target nucleic acids, for which a reference nucleic acid sequence is known, present in one or more biological samples.

102. [currently amended] A kit for ~~sequence analysis~~ mass spectrometry sequencing according to a method of claim 57 of one or more unknown target nucleic acids in one or more biological sample using mass spectroscopy, the kit comprising:

- (a) one or more nucleotide triphosphates;
- (b) one or more polymerases; and,
- (c) one or more nucleic acid cleaving agents;
- (d) ~~optionally~~, reagents to purify the target nucleic acid;
- (e) ~~optionally~~, ion exchange beads in order to purify the non-ordered set of fragments;
- (f) ~~optionally~~, a solid support suitable for use in mass spectrometry analysis whereon the non-ordered set of fragments may be spotted; and,
- (g) ~~optionally~~, computer software for analysing the mass spectra of the sequence of said target nucleic acid resulting in one or more unique sequences.

103. [canceled] Use of a kit according to claim 102 for analyzing the sequence of, for determining the sequence of, or, for performing genome wide genotyping using one or more target nucleic acids of unknown sequence present in one or more biological samples.

104. [new] The method of claim 84, wherein the pyrimidine-specific RNase is RNase A.

105. [new] The method of claim 57 or 89, wherein said method comprises four RNase-specific cleavage reactions.

106. [new] The method of claim 105, wherein said four RNase-specific cleavage reactions comprise RNase T1 and RNase U2 cleavage of the + and – strands of said target nucleic acid.

107. [new] The method of claim 105, wherein said four RNase-specific cleavage reactions comprise RNase A or RNase A and RNase T1 cleavage of the + and – strands of said target nucleic acid.

108. [new] The kit of claim 100 wherein said cleaving agent is an endonuclease selected from the group consisting of U/C specific RNase A, G-specific T1 ribonuclease, A-specific U2 ribonuclease, A/U specific phyM ribonuclease, C-specific chicken liver ribonuclease (RNaseCL3) and cusativin.

109. [new] The kit of claim 102 wherein said cleaving agent is an endonuclease selected from the group consisting of U/C specific RNase A, G-specific T1 ribonuclease, A-specific U2 ribonuclease, A/U specific phyM ribonuclease, C-specific chicken liver ribonuclease (RNaseCL3) and cusativin.

110. [new] The kit of claim 100 or 102, wherein said one or more polymerases are SP6 and T7 RNA polymerase and said cleaving agent is an endonuclease selected from the group consisting of U/C specific RNase A, G-specific T1 ribonuclease, A-specific U2 ribonuclease, A/U specific phyM ribonuclease, C-specific chicken liver ribonuclease (RNaseCL3) and cusativin.

111. [new] The kit of claim 100, wherein said kit comprises:

- (a) four nucleotide triphosphates;
- (b) a T7 or SP6 polymerase;
- (c) a RNase T1 and RNase U2;
- (d) one or more sets of reference nucleic acids for which the nucleic acid sequence is known;
- (e) reagents to purify the target nucleic acid;
- (f) ion exchange beads in order to purify the non-ordered set of fragments;

- (g) a solid support suitable for use in mass spectrometry analysis whereon the non-ordered set of fragments may be spotted; and
- (h) a computer software for comparing the mass spectra of the one or more target nucleic acid with the mass spectra of the reference nucleic acid and deducing therefrom the nucleic acid sequence of the target nucleic acid.

112. [new] A kit of claim 102, wherein said kit comprises:

- (a) four nucleotide triphosphates;
- (b) a T7 or SP6 polymerase;
- (c) a RNase T1 and RNase U2;
- (d) reagents to purify the target nucleic acid;
- (e) ion exchange beads in order to purify the non-ordered set of fragments;
- (f) a solid support suitable for use in mass spectrometry analysis whereon the non-ordered set of fragments may be spotted; and,
- (g) a computer software for analysing the mass spectra of the sequence of said target nucleic acid resulting in one or more unique sequences.

113. [new] The kit of claim 111 or 112, wherein said T7 or SP6 polymerase is a mutant polymerase that incorporates non-canonical substrates with a 2'-deoxy, 2'-O-methyl, 2'-fluoro or 2'-amino substituent into the transcript.

114. [new] A kit for mass spectrometry sequencing according to a method of claim 57 or claim 89 of one or more target nucleic acids in one or more biological samples using mass spectrometry, the kit comprising:

- (a) one or more ribonucleotide triphosphates and one or more deoxyribonucleotide triphosphates;
- (b) one or more polymerases;
- (c) one or more RNAses; and
- (d) a solid support suitable for use in mass spectrometry analysis whereon the non-ordered set of fragments may be spotted.

REMARKS

I. Status of the Claims

Claims 57-103 are pending in the instant application and stand variously rejected under 35 U.S.C. §101, 35 U.S.C. §112 second paragraph as being indefinite for failing to particularly point out and distinctly claim the invention, and under 35 U.S.C. §102(b) and 35 U.S.C. §103. Applicants respectfully traverse the rejections and request reconsideration in light of the above amendments and the following remarks.

Claims 104-112 are newly presented herein by amendment and are fully supported by the specification as filed. For example, new claim 104 is directed to a specific embodiment in which the pyrimidine-specific RNase of claim 84 is RNase A. This new claim is supported at *e.g.*, page 9, line 20. New claims 105, 106 and 107 are directed to a specific embodiment of the method of claim 57 or claim 89 in which there are four RNase-specific cleavage reactions. The exemplary four reaction in claim 106 are RNase T1 and RNase U2 cleavage of the + and – strands of the target nucleic acid. These claims are supported by the specification *e.g.*, at page 39, lines 1-5. The exemplary four reactions in claims 107 are RNase A or RNase A and RNase T1 cleavage of the + and – strands of said target nucleic acid. This claim is supported by the disclosure at page 23, line 21 through page 24, line 6. Claims 108-110 provide specific embodiments of the kits of the present invention in which the cleavage reagent is defined as an exonuclease, and these claims are supported *e.g.*, at page 9 lines 16-24 of the specification. New claims 111-113 are specific kits that comprise particular polymerase and the cleavage reagents are supported by the specification at page at page 8, lines 23-26 and page 9 lines 16-24. New claim 114 is directed to specific kits that comprise ribo- and deoxyribonucleoside triphosphates, at least one polymerase, at least one RNase and a solid support for use in mass spectrometry analysis. This claim is supported by the specification page 23, line 21 through page 24, line 6. Applicants respectfully request entry of these new claims in the instant application.

II. Rejection under 35 U.S.C. §101 should be withdrawn

Claims 92-95, 97-99, 101, and 103 stand rejected under 35 U.S.C. §101 for recitation of a “use,” which allegedly does not comport with statutory subject matter. Applicants have canceled claims 101 and 103 by amendment herein above, thereby obviating the rejection of those claims. Applicants have amended claims 92-95 and 97-99 as “methods” claims. Applicants believe that this amendment obviates the grounds for rejection and applicants respectfully request that the rejection of claims 92-95 and 97-99 under 35 U.S.C. §101 be withdrawn.

III. Rejection under 35 U.S.C. §112, second paragraph should be withdrawn

Claims 61-66 and 84 were rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to point out and distinctly claim the subject matter which applicants regard as the invention.

More specifically, claim 61, and therefore claims 62-66, which depend from claim 61, was rejected for being dependent from non-existing claim 4. Applicants have corrected this typographical error in the above amendment and made claim 61 dependent from claim 60. Applicants believe this amendment addresses clarifies the claim and obviates the grounds for rejection.

Claim 84 was rejected for recitation of the term “such as” which assertedly rendered the claim indefinite. Applicants have amended the claim to remove the phrase “such as RNase A.” This clarifies claim 84. Applicants have presented new claim 104 in which this exemplary RNase is presented for use in the claimed method. New claim 104 is fully supported by the specification as filed, for example at page 9, line 20.

Applicants submit that the above amendments and comments address all of the rejections based on 35 U.S.C. §112, second paragraph and request that the rejections be withdrawn in light of these comments.

IV. Rejection under 35 U.S.C. §102(b), should be withdrawn

Claims 57-71, 73-80, 82-83, 85-91 and 96 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Monforte et al (WO 97/33000). Claims 100-103 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by the disclosure of New England BioLabs Catalog (Product Number 203S and 203L, page 74, 1996-1997. Applicants respectfully traverse both rejection and request reconsideration in view of the present remarks

a. Claims 57-71, 73-80, 82-83, 85-91 and 96

The Examiner asserts that the Monforte document "teaches a method of sequence analysis." However, Applicants respectfully disagree with the Examiner's characterization of the Monforte document.

Monforte uses nonrandom fragmentation technique(s) for the *detection* of sequence variations in a target nucleic acid relative to reference (*i.e.* wild type) nucleic acid. A target nucleic acid is typically too large to analyze directly (current mass spectrometers do not have the required accuracy and resolution) and, consequently, the target must be fragmented (see Monforte, page 17, lines 8-12). Monforte expressly states that the methods described in that publication ***do not*** involve sequencing of a target nucleic acid. For example, Applicants refer to page 10, lines 2-6, where Monforte begins the Summary of their invention explicitly disclaiming that the invention described therein:

"provides methods of and kits for detecting mutations in a target nucleic acid comprising nonrandomly fragmenting said target nucleic acid to form a set of nonrandom length fragments (NLFs), determining the masses of members of said set of NLFs using mass spectrometry, ***wherein said determining does not involve sequencing of said target nucleic acid.***"

Monforte's methods involve the preparation of single-stranded, nonrandom length fragments of a target nucleic acid and analysis of the mass of each of the fragments. The fragments are derived from one of either the positive or the negative strand or from both the positive and negative strands (Page 28 lines 5-17). The Examiner referred Applicants to

Figures 2-13 of the Monforte document. From these figures, it appears that Monforte uses source nucleic acid and amplifies it to produce the target. The target is then treated with restriction endonucleases to produce all the NLFs. The NLFs are then purified and the mass of the fragments is determined by MS analysis. (FIG 2). FIG 3 describes a variation of the technique in which there is a heterozygous mix of wild-type and mutant in the target and the masses of the fragments from these two targets are compared. But in each of the figures and the disclosure *there is no indication* that any of the mass spectrometry data is used to compile the sequence of the target and recompile the initial target sequence. In fact, as discussed above, Monforte expressly states that the technique “*does not involve sequencing*” of the target nucleic acid.

Using the above described methods, Monforte is able to get some limited information “about the nature and location of the mutation in the target nucleic acid” (page 16, lines 24-25) because a shift in the mass of an NLF is indicative of the presence of a mutation. However, this information is indeed limited because, at best, using this information one can only “localize the region containing the mutation” by using overlapping fragments (page 35, lines 20-30). Thus, while it might be possible to use Monforte to show that there is “a difference” between a wild-type and a mutant sequence. It would not be possible to determine the exact sequence being observed. Furthermore, the position of the presumed mutation cannot be unambiguously determined because the artisan cannot tell exactly which nucleic acid is being replaced by which other nucleic acid residue. Moreover, when the observed mass shift is consistent with a previously known mutation, one cannot be absolutely sure that it is that exact mutation which is present in the sample nucleic acid of interest.

By contrast, the present claims are directed to “a method of sequence analysis of one or more target nucleic acids,” *i.e.*, the present methods are directed to sequencing or re-sequencing the target nucleic acid. The term “sequence analysis” as used in claims 57 and 89, is meant to refer to sequencing or re-sequencing (respectively) of a given target nucleic acid, and not merely an relative comparison of two sequences, which is what is performed by

Monforte. In order to clarify that the claims 57 and 89 are directed to sequencing and re-sequencing respectively, Applicants have amended the preamble of each of these claims.

Thus, as amended, the claims of the present invention are directed to sequencing a given target nucleic acid (e.g., claim 57), or re-sequencing a nucleic acid to confirm its sequence (e.g., claim 89). In both of these aspects, the nucleotide sequence of the target nucleic acid is determined. These techniques provide an alternative to the conventional sequencing techniques available in the art. The techniques of the present invention use a "combination of several different mass spectra, obtained after complementary digestion reactions" (page 4, lines 28-29). Thus, in the presently claimed invention, the products of multiple cleavage reactions of a target nucleic acid are subjected to mass spectrometry (Applicants have clarified claims 57 and 84 to recite that there are two or more cleavage reactions in the technique, this amendment is supported e.g., page 38, lines 29-30). Subsequently, the information from the various mass spectra is integrated to result in an unambiguous sequence analysis, *i.e.* the combination of mass shifts and/or changes in cleavage pattern observed in the mass spectra results in the unequivocal determination of the sequence (variations).

Thus, the independent claim of the invention (claim 57) describe a technique that not only detects a mutation but, additionally, gives the target sequence and thus allows one to determine the nature of the mutation, as well as, its location within the target nucleic acid sequence. As such, the method of the independent claim is novel over the disclosure of Monforte, which not only fails to teach sequencing of a target nucleic acid, but goes on to explicitly state that its methods ***do not*** involve sequencing of the target nucleic acid. As the independent claims are novel, Applicants respectfully submit that dependent claims 58-71, 73-80, 82-83, 85-91 and 96, which depend from the independent claims also are novel for at least the reasons given for the independent claim.

In light of the above comments, Applicants submit that it is clear that the Monforte document does not teach the sequencing methods described in the present invention. As such, Applicants respectfully request that the rejection of claims 57-71, 73-80,

82-83, 85-91 and 96 under 35 U.S.C. §102(b) based on Monforte be withdrawn and the claims be reconsidered for allowance.

b. Claims 100-103

Claims 100-103 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by New England BioLabs Catalog (Product Number 203S and 203L, page 74, 1996-1997), which is purported to teach one or more nucleotide triphosphates, one or more polymerases, one or more nucleic acid cleaving agents and one or more sets of reference nucleic acids for which the nucleic acid sequence is known and optionally reagents to purify the target nucleic acid. Claims 101 and 103 have been canceled herein by amendment but nonetheless Applicants respectfully traverse the rejection with respect to all the kit claims.

The kits of the present invention are kits for use in the mass spectrometry analysis of nucleic acids. Applicants submit that the New England BioLabs Catalog disclosure associated with product numbers #203S and #203L is not directed to mass spectrometry kits that can be used to sequence a target nucleic acid, as is required of the kits of the present invention. Moreover, the kits of claims 100 and 102 also expressly require “a solid support suitable for use in mass spectrometry analysis,” “ion exchange beads in order to purify the non-ordered set of fragments” and further contain “software for analyzing (or comparing) the mass spectra” of the nucleic acids. The disclosure cited by the Examiner does not provide a teaching of these latter three components of the kits of the present invention. In the absence of these teachings, the disclosure cannot be said to anticipate the claimed kits of the present invention. Therefore, Applicants respectfully request that the rejection of claims 100-102 be withdrawn and the claims be reconsidered for allowance.

V. Rejection under 35 U.S.C. §103(a), should be withdrawn

Claim 72 was rejected under 35 U.S.C. §103 as allegedly being obvious over Monforte in view of Geysen (U.S. Patent No. 6,475,807). Claim 81 was rejected under 35 U.S.C. §103 as allegedly being obvious over Monforte in view of Hanna (U.S. Patent No. 6,107,039). Claim 84 stands rejected under 35 U.S.C. §103 as allegedly being obvious over

Monforte in view of the New England BioLabs catalog product numbers #251L and 207L, (page 75 1996). Applicants respectfully traverse these rejections.

In order to establish a *prima facie* case of obviousness the cited art must teach each element of the claimed invention. Moreover, where multiple references are used, there must be a suggestion or motivation to combine those references to arrive at the teachings of the invention and there must be a reasonable expectation of successfully realizing the invention in view of the teachings and the state of the art. *In re Vaeck*, 20 USPQ2d 1438, 1445 (Fed. Cir. 1991). These teachings must arise out of the prior art and not from the Applicants' own disclosure. As stated in MPEP 2143, these criteria must be met in order to properly establish *prima facie* obviousness. It is the Applicants' position that these criteria are not met by the teachings cited by the Examiner in the present case, and as such a *prima facie* case of obviousness has not been established.

For each of the rejections (*i.e.*, the rejection of claim 72, claim 81 and claim 84) the Examiner relies on the disclosure of Monforte as a primary reference as generally teaching the methods of the invention claimed in claims 57-71, 73-80, 82-83, 85-91 and 96. However, as explained above, Applicants respectfully submit that the Examiner is incorrect in his assertions because the Monforte document does not teach a method of sequencing as claimed herein but rather is directed to a simple detection and comparison method. In fact, the Monforte document expressly states that its method involves "determining the masses of members of said set of NLFs using mass spectrometry, ***wherein said determining does not involve sequencing of said target nucleic acid.***" Moreover, Monforte states that nucleic acids typically are too large to analyze directly because current mass spectrometers do not have the required accuracy and resolution to identify a single base change in molecules and, consequently, the target must be fragmented (see page 17, lines 8-12) As such, reviewing the Monforte disclosure as a whole, one of skill in the art would be dissuaded from attempting to use mass spectrometry analysis for sequencing of a target nucleic acid, seeing as this was a technique explicitly disclaimed by Monforte. With the above key to the differences and patentability of the instant claim over Monforte in mind, Applicants move on to discuss each of the combinations cited by the Examiner.

a. **Claim 72 is non-obvious over the Monforte/Geysen combination.**

Claim 72 is non-obvious over the Monforte/Geysen combination cited by the Examiner. Claim 72 is dependent from claim 67, which is in turn is dependent from main claim 57. Thus, claim 72 incorporates all of the limitations of the method of claim 57. As discussed above, the method of claim 57, *i.e.*, a method of sequencing a target nucleic acid using mass spectrometry by subjecting a target nucleic to two or more separate base-specific, sequence-specific or site-specific complementary cleavage reactions, wherein each cleavage reaction generates a non-ordered set of fragments and subsequently using mass spectrometry and computational analysis to determine the sequence of the target nucleic acid. This claim as discussed above is novel and non-obvious over the cited art. Claim 72 is an embodiment of the novel and non-obvious method of claim 57, which *further includes* the limitation that the target nucleic acid has been modified to consist of nucleotides that incorporate alternative isotopes.

Adding the Geysen disclosure to Monforte *does not* overcome Monforte's teaching away from the sequencing aspects of the instant claims. As discussed above, Monforte completely fails to suggest or motivate one of skill in the art to sequence target nucleic acids using mass spectrometry. Indeed, Monforte teaches the exact opposite, namely, that Monforte's methods "does not involve the sequencing of said target nucleic acid." Geysen does nothing to rehabilitate Monforte with and appropriate suggestion/motivation to sequence the target nucleic acid. The disclosure of Geysen is directed to "insertion of isotopically labeled portions into solid state combinatorial synthesis constructs followed by mass spectrometer, mass-based nuclear magnetic resonance spectrometry or mass-based infrared spectrometry analysis allows for the physical, non-chemical encoding of large numbers of combinatorial synthesis products." (see abstract of Geysen).

The Geysen disclosure is merely directed to methods of "tagging" a given component of a combinatorial library with a radioisotope to make it amenable to detection. The Geysen disclosure neither suggests or teaches how such tags might be used to sequence a length of target nucleic acid. Thus, the combination of Monforte with Geysen remains

flawed for the purposes of obviousness because there is no suggestion or motivation in either reference that it would be desirable to use the combined techniques of these two documents to sequence a target nucleic acid. In fact, the suggestion is the opposite, *i.e.*, to detect a target nucleic acid using the mass spectrometry methods of Monforte using a label of Geysen but *without* actually sequencing the target nucleic acid.

Moreover, even if the skilled artisan were to fortuitously combine Monforte with Geysen, that individual would not have any expectation of success of achieving the sequencing/re-sequencing methods claimed in the present invention. At best, the combination of Geysen with Monforte will yield a method in which Monforte, *without sequencing the target nucleic acid*, manages to detect a nucleic acid mutation in the target nucleic acid where the target nucleic acid is labeled with an isotope using a labeling method of Geysen. There is no reason to believe that one of skill would be able to derive the nucleotide sequence the target nucleic acid from this combination of references. The only expectation of success of sequencing the target nucleic acid arises first and foremost out of the disclosure of the instant specification, and use of the instant specification to reconstruct the claimed methods out of the combination of Monforte and Geysen, amounts to an impermissible hindsight reconstruction of the claimed invention.

In light of the above comments, Applicants respectfully submit that claim 72 is non-obvious over the combination of Monforte and Geysen and Applicants request that the rejection be withdrawn.

b. Claim 81 is non-obvious over the Monforte/Hanna combination

Applicants submit that the rejection of claim 81 under 35 U.S.C. § 103(a) over a combination of Monforte with Hanna also is flawed for the following reasons and respectfully request that the rejection be withdrawn. Claim 81 is dependent from claim 57, and further defines the claimed method of claim 57 in that the “target nucleic acids are phosphorothioate-modified single stranded DNA or RNA, and wherein the cleavage reactions are performed with the nuclease P1.” The above discussion of the non-obviousness of the method of claim 57 over Monforte is incorporated into the instant section. As discussed

above Monforte fails to teach a mass spectrometry-based nucleic acid sequencing method. In order for Hanna to properly rehabilitate Monforte, Hanna must not only teach one of skill in the art that it would be possible or desirable to modify a target nucleic to make the nucleic acid a phosphorothioate-modified single stranded DNA or RNA and the use of nuclease P1 cleavage, *but* Hanna must also provide a teaching or suggestion that such a modification and cleavage should be used to sequence the target nucleic acid using mass spectrometry. Hanna simply fails to meet this hurdle.

Hanna is directed to methods and compositions directed to using protected thiol analogs of pyrimidine bases. These thiol analogs can be incorporated into oligonucleotides or polynucleotides, deprotected and derivatized with a functional group. Thus, Hanna is directed to synthesizing oligonucleotides with derivatized functional groups. And while Hanna also does describe P1 based digestion of nucleic acids, Hanna fails to present any teaching of determining the sequence of a target nucleic acid. In the absence of this teaching, combining Hanna with Monforte will *only* produce a method of *detecting* a target nucleic acid containing one of Hanna's analogs using the mass spectrometry method of Monforte, but the combination will still fail to teach how to sequencing the target nucleic acids. Thus, for reasons analogous to those given for the Monforte/Geysen combination above, the combination of Monforte with Hanna fails to establish a *prima facie* case of obviousness of claim 81. As such, Applicants respectfully request that the rejection be withdrawn.

c. Claim 84 is non-obvious over the Monforte/New England BioLabs catalog combination

Analogously to the discussion above, a combination of Monforte with the New England BioLabs catalog page 75 (1996) also fails to establish *prima facie* obviousness of claim 84 and, therefore, the rejection should be withdrawn. The drawbacks of the Monforte disclosure are discussed above. The New England BioLabs catalog only provides a description of the T7 and SP6 RNA polymerases and their use in the synthesis of "labeled single stranded RNA transcripts of high specificity." The disclosure provides no teaching,

suggestion or motivation to one of skill in the art that such polymerases could or should be used in conjunction with a mass spectrometry method to determine the nucleotide sequence of a target nucleic acid. In the absence of such a teaching, suggestion or motivation, this catalog page fails to overcome the flaws in the Monforte disclosure with respect to the alleged obviousness of claim 84. Not only would one of skill in the art not be motivated to perform the method of claim 84 from the combination of Monforte with the New England BioLabs catalog, one of skill also would not have a reasonable expectation of success of achieving sequencing of a target nucleic acid because Monforte itself suggests the exact opposite teaching, *i.e.*, not sequencing the target nucleic acid.


In light of the above comments, Applicants respectfully submit that claims 71, 82 and 84 are non-obvious over the cited art and that the rejections articulated by the Examiner at pages 8-12 of the Office Action should be withdrawn.

VI. Conclusions

Applicants believe that all of the rejections have been overcome and the claims of the instant application are now in condition for allowance and request an early indication of such a favorable disposition of the case. The Examiner is invited to contact the undersigned with any questions, comments or suggestions relating to the referenced patent application.

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Respectfully submitted,

By 

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